

# Intrinsic Characteristics of the Proton Pump in the Luminal Membrane of a Tight Urinary Epithelium

## *The Relation Between Transport Rate and $\Delta\tilde{\mu}_H$*

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**ABSTRACT** A number of tight urinary epithelia, as exemplified by the turtle bladder, acidify the luminal solution by active transport of  $H^+$  across the luminal cell membrane. The rate of active  $H^+$  transport ( $J_H$ ) decreases as the electrochemical potential difference for  $H^+$  [ $\Delta\tilde{\mu}_H = \tilde{\mu}_H(\text{lumen}) - \tilde{\mu}_H(\text{serosa})$ ] across the epithelium is increased. The luminal cell membrane has a low permeability for  $H^+$  equivalents and a high electrical resistance compared with the basolateral cell membrane. Changes in  $J_H$  thus reflect changes in active  $H^+$  transport across the luminal membrane. To examine the control of  $J_H$  by  $\Delta\tilde{\mu}_H$  in the turtle bladder, transepithelial electrical potential differences ( $\Delta\psi$ ) were imposed at constant acid-base conditions or the luminal pH was varied at  $\Delta\psi = 0$  and constant serosal  $P_{CO_2}$  and pH. When the luminal compartment was acidified from pH 7 to 4 or was made electrically positive,  $J_H$  decreased as a linear function of  $\Delta\tilde{\mu}_H$  as previously described. When the luminal compartment was made alkaline from pH 7 to 9 or was made electrically negative,  $J_H$  reached a maximal value, which was the same whether the  $\Delta\tilde{\mu}_H$  was imposed as a  $\Delta\text{pH}$  or a  $\Delta\psi$ . The nonlinear  $J_H$  vs.  $\Delta\tilde{\mu}_H$  relation does not result from changes in the number of pumps in the luminal membrane or from changes in the intracellular pH, but is a characteristic of the  $H^+$  pumps themselves. We propose a general scheme, which, because of its structural features, can account for the nonlinearity of the  $J_H$  vs.  $\Delta\tilde{\mu}_H$  relations and, more specifically, for the kinetic equivalence of the effects of the chemical and electrical components of  $\Delta\tilde{\mu}_H$ . According to this model, the pump complex consists of two components: a catalytic unit at the cytoplasmic side of the luminal membrane, which mediates the ATP-driven  $H^+$  translocation, and a transmembrane channel, which mediates the transfer of  $H^+$  from the catalytic unit to the luminal solution. These two components may be linked through a buffer compartment for  $H^+$  (an antechamber).

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## INTRODUCTION

The turtle urinary bladder secretes protons actively into the urinary compartment (Steinmetz, 1974). The proton pump is tightly coupled to cell metabolism (Beauwens and Al-Awqati, 1976) and is probably a proton-translocating ATPase (Dixon and Al-Awqati, 1979; Steinmetz et al., 1981) located in the luminal membrane of the carbonic anhydrase (CA)-containing cells in the epithelium (Schwartz et al., 1972). The pump transports only one ion species (Steinmetz, 1967, 1974; Steinmetz and Andersen, 1982) and is electrogenic with a single charge transfer step (class-I pump, Hansen et al., 1981).

The proton transport rate depends upon several factors: first, the cellular acid-base status, which determines the luminal membrane area of the CA cells and with it the number of proton pumps in the membrane (Husted et al., 1981; Gluck et al., 1982; Stetson and Steinmetz, 1983); second, the supply of protons and metabolic energy to the pumps (Schwartz and Steinmetz, 1977; Cohen et al., 1978); third, the transepithelial electrochemical potential difference for protons,  $\Delta\tilde{\mu}_H = \tilde{\mu}_H(\text{lumen}) - \tilde{\mu}_H(\text{serosa})$  (Steinmetz and Lawson, 1971; Al-Awqati et al., 1977).

The relation between the active proton transport rate,  $J_H$ , and the transepithelial pH difference,  $\Delta\text{pH}$ , or potential difference,  $\Delta\psi$ , is linear in a variety of experimental conditions (Al-Awqati et al., 1977). Similar linear relations observed for other active transport systems have led to the formulation of equivalent circuit descriptions for these transport systems, in which the pumps are depicted as an electromotive force in series with a resistance (Ussing and Zerahn, 1952; Helman and Thompson, 1982). The relationship between  $J_H$  and  $\Delta\tilde{\mu}_H$  reflects, however, not only the intrinsic characteristics of the pumps but also cellular factors, such as the distribution of pumps between the luminal membrane and the cytoplasmic vesicles that contain a reserve of pumps, as well as transport limitations imposed by the basolateral cell membrane. Attempts to model active transport systems kinetically (e.g., Hansen et al., 1981; Steinmetz and Andersen, 1982) indicate furthermore that the linear relation between  $J_H$  and  $\Delta\tilde{\mu}_H$  should hold only over a limited range of  $\Delta\tilde{\mu}_H$ , and that  $J_H$  should approach saturating values at either extreme of  $\Delta\tilde{\mu}_H$ . Experimentally,  $J_H$  was found to reach a saturating value when the cellular  $\text{CO}_2$  tension or  $\text{H}^+$  concentration was increased (Cohen and Steinmetz, 1980).

The present study was undertaken to explore the relation between  $J_H$  and  $\Delta\tilde{\mu}_H$  over a wide range of  $\Delta\tilde{\mu}_H$  ( $\Delta\text{pH}$  or  $\Delta\psi$ ), under conditions where the complications introduced by the series resistance imposed by the basolateral cell membrane and by changes in the number of pumps in the luminal cell membrane could be disregarded. It was thus possible to regard the complex epithelium as being equivalent to a single membrane, the luminal cell membrane, and to define some of the intrinsic characteristics of the proton pumps.

## METHODS

Urinary bladders of freshwater turtles, *Pseudemys scripta elegans* (W. Lemberger and Co., Oshkosh, WI), were mounted in Lucite chambers with an exposed surface area of  $8 \text{ cm}^2$ , as previously described (Steinmetz, 1967). The bladders were generally maintained in the

short-circuit state by voltage-clamping. The exceptions were in the series of experiments where  $J_H$  was studied as a function of the transepithelial potential difference. The two sides of the bladders were initially bathed with identical Ringer's solutions containing 114.4 mM NaCl, 3.5 mM KCl, 2.0 mM  $\text{Na}_2\text{HPO}_4$ , 0.5 mM  $\text{MgCl}_2$ , 1.8 mM  $\text{CaCl}_2$ , and 5.0 mM glucose. The serosal solution contained, in addition,  $5 \times 10^{-4}$  M ouabain. The mucosal solution was gassed with  $\text{CO}_2$ -free air, and the serosal solution was gassed with 1%  $\text{CO}_2$  in air (except for one series of experiments in which both solutions were gassed with  $\text{CO}_2$ -free air). The aim of this protocol was to provide enough  $\text{CO}_2$  to support proton transport without introducing a significant  $\text{HCO}_3^-$  flux across the epithelium. (This level of  $\text{CO}_2$  and  $\text{HCO}_3^-$  has previously been shown to have a negligible effect on our ability to measure the active  $\text{H}^+$  transport rate [Leslie et al., 1973; Schwartz, 1976].) The pH of the serosal bulk solution was kept constant at  $6.8 \pm 0.1$ , while the pH of the mucosal solution was varied as indicated in the text.<sup>1</sup>

The rate of proton secretion was measured as the (reverse) short-circuit current after active  $\text{Na}^+$  transport was abolished by the addition of ouabain to the serosal solution (Schwartz, 1976). In the experiments where a transepithelial potential difference was imposed, the rate of active  $\text{H}^+$  secretion was also measured by pH-stat titration (Steinmetz, 1967).

Results are reported as transport rate (mean  $\pm$  SEM) per  $8 \text{ cm}^2$  bladder surface, which represents a dry tissue weight of  $\sim 14 \text{ mg}$ .

## RESULTS

### *The Relation Between $J_H$ and Luminal pH*

Fig. 1 is a tracing of the proton current ( $J_H$ ) measured under short-circuit conditions as a function of the luminal pH. The luminal pH was first lowered from 7.1 to 4.4, which reduced  $J_H$  to a value near zero. The pH was then increased by increments of 0.5–0.7 pH units, which resulted in stepwise increases in  $J_H$ , and  $J_H$  was restored by returning the pH to 7.3. Further increases in the luminal pH resulted in much smaller increases of  $J_H$ , which suggests that the active proton transport rate is a "saturating" function of the luminal pH (transepithelial  $\Delta\text{pH}$ ). Acidification of the luminal compartment to pH 4.5 again decreased  $J_H$  to about zero (Fig. 1, toward the right). This decrease was rapidly and completely reversed when the luminal pH was returned to 8.5.

The relation between luminal pH and  $J_H$  has traditionally been considered to be determined by energetic and kinetic factors operating on each  $\text{H}^+$  pump at the luminal membrane. However,  $J_H$  can also be regulated by fusion of cytoplasmic vesicles into the luminal membrane of the CA cells in the turtle urinary bladder, thus increasing the number of pumps, and by endocytotic retrieval of the luminal membrane into cytoplasmic vesicles, thus decreasing the number of pumps (Husted et al., 1981; Gluck et al., 1982; Stetson and Steinmetz, 1983). Variations of  $J_H$  as a function of luminal pH (transepithelial  $\Delta\text{pH}$ ) may thus reflect variations in the number of  $\text{H}^+$  pumps, as well as energetic and kinetic factors. We have attempted to distinguish between these possibilities by examining, first, the time course of  $J_H$  following sudden changes in luminal pH, and

<sup>1</sup> The intracellular pH under these conditions is thought to be near 7.4 as judged from studies with dimethylxazolidione (Steinmetz, 1969; Cohen and Steinmetz, 1980).

second, how the relation between  $J_H$  and luminal pH is affected by colchicine, an inhibitor of microtubular function that has been found to inhibit exocytosis and the associated membrane fusion of cytoplasmic vesicles (e.g., Gluck et al., 1982; Palmer and Lorenzen, 1983; Stetson and Steinmetz, 1983).

*The  $J_H$  vs.  $\Delta pH$  Relation Is Not Determined by Changes in the Number of Pumps*

The effects of large repetitive changes in luminal pH are shown in Fig. 2. The maximal transport rates at pH 8.4 remained the same after luminal pH changes to between 4.1 and 4.5, during which  $J_H$  was reduced to near zero. The fairly rapid transitions and the reproducibility of the transport rates at the two pH extremes argue that the changes in  $J_H$  result mainly from the energetic and kinetic consequences of the changes in the luminal pH (transepithelial  $\Delta pH$ ).

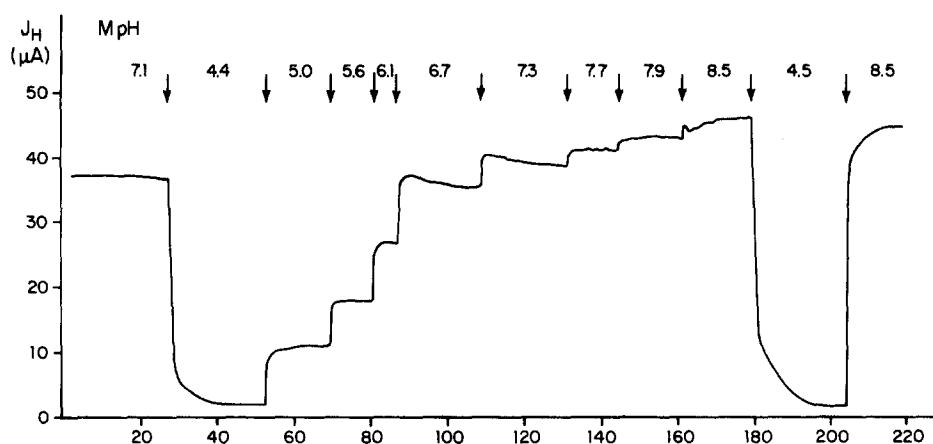


FIGURE 1. Tracing of  $J_H$  as a function of luminal pH (M pH). This experiment is representative of eight experiments done under similar conditions. See text for details.

The magnitude of the response was independent of the past history of the bladder and vesicle reserve in the cytoplasm.<sup>2</sup>

It is important that the shape of the relation between  $J_H$  and luminal pH was unaffected by colchicine at a concentration sufficient to abolish the membrane fusion events ( $10^{-4}$  M, Gluck et al., 1982). Fig. 3 shows an experiment similar to that illustrated in Fig. 1, except that the bladder had been exposed to  $10^{-4}$  M colchicine for 2 h before the start of the experiment.

Stepwise decreases in luminal pH were again associated with stepwise decreases in  $J_H$ , which reached zero at a luminal pH of 4.7. The subsequent increase of the luminal pH to 8.6 led to a rapid increase in  $J_H$  to the maximal level. The pH dependence of  $J_H$  was measured in six experiments in which the luminal pH was increased 2 pH units, from pH  $4.5 \pm 0.2$  (where  $J_H = 0$ ).  $J_H$  increased from 0 to

<sup>2</sup> The time courses of the vesicle fusion and membrane retrieval events are not known in detail. Vesicle fusion appears to be quite rapid, while membrane retrieval seems to be slower process, with a half-time of ~10–30 min (Al-Awqati, Q., personal communication).

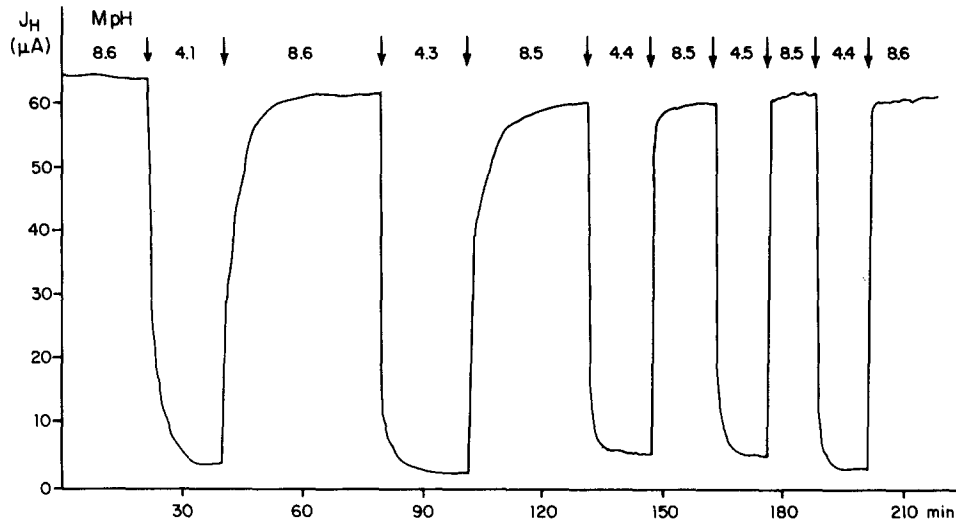


FIGURE 2.  $J_H$  as a function of large repetitive changes in luminal pH (M pH).

$32 \pm 7 \mu\text{A}$  in the colchicine-treated bladders and from 0 to  $42 \pm 11 \mu\text{A}$  in the control bladders.

#### *The Acid-Base Permeability of the Basolateral Cell Membrane*

The acute changes in  $J_H$  following sudden changes in luminal pH were further characterized in the absence of exogenous  $\text{CO}_2$  (and  $\text{HCO}_3^-$ ), i.e., under conditions where the extrusion of  $\text{OH}^-$  (in the form of  $\text{HCO}_3^-$ ) across the basolateral

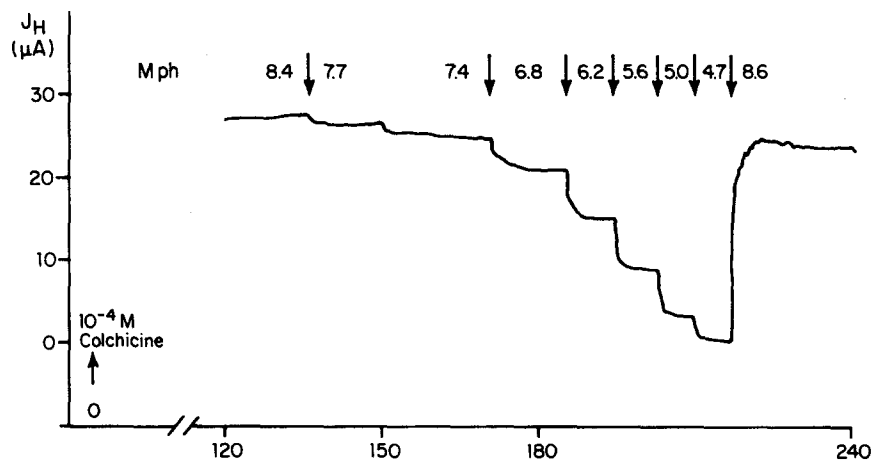


FIGURE 3. Tracing of  $J_H$  as a function of luminal pH (M pH) in a bladder pretreated for 120 min with  $10^{-4}$  M colchicine. Note the rapid recovery of  $J_H$  after M pH was restored from 4.7 to 8.6. Although colchicine inhibits the stimulation of  $J_H$  by  $\text{CO}_2$ , it causes only minor inhibition of  $J_H$  at a constant  $P_{\text{CO}_2}$  (Stetson and Steinmetz, 1983), and the  $J_H$  vs.  $\Delta\text{pH}$  relation was preserved in the presence of colchicine.

cell membrane is decreased, and the stationary  $H^+$  transport rate is reduced (Schwartz and Steinmetz, 1971; Al-Awqati et al., 1977). In this case, it was found that  $J_H$  exhibited a pronounced biphasic behavior (Fig. 4). The stationary  $J_H$  at pH 6.8 was reduced to ~50% of the control value seen with exogenous  $CO_2$  (1%) (and a  $[HCO_3^-]$  of ~1 mM).

$J_H$  decreased to near 0  $\mu A$  when the luminal pH was reduced to 4.9, but the subsequent pH increase to 8.2 did not lead to the maintained increase in  $J_H$  seen in Fig. 2. Instead,  $J_H$  increased to a level consistent with that seen in the presence of  $CO_2$  and declined thereafter to a level only slightly greater than that seen at pH 6.9. The overshoot in  $J_H$  is interpreted to result from an increase in the

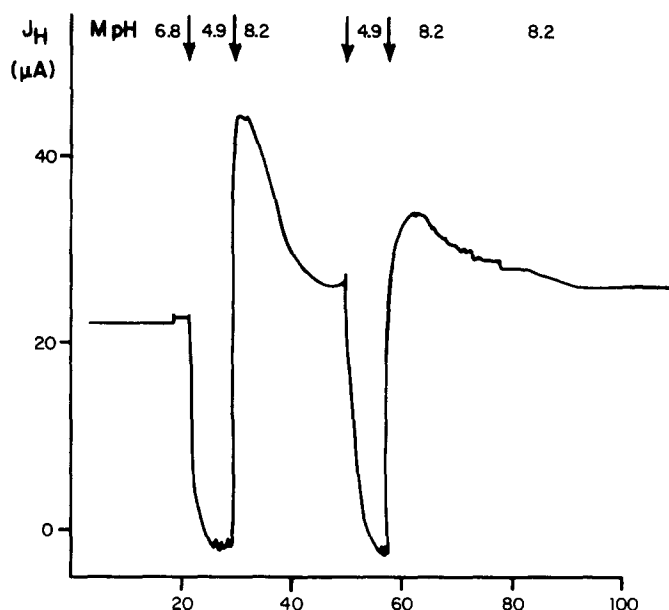


FIGURE 4. Tracing of the acute changes in  $J_H$  induced by sudden changes in luminal pH (M pH) in the absence of exogenous  $CO_2$  and  $HCO_3^-$ . Note the overshoot in  $J_H$  when M pH is increased from 4.9 to 8.2.

acidity of the  $H^+$ -secreting cells, which occurred during the period in which the pump was stopped by the  $\Delta pH$ . Removal (or reversal) of the  $\Delta pH$  should lead to an increase in  $J_H$  to a level determined by the cell  $[H^+]$  at the time at which the luminal pH was changed. If, however, the luminal  $H^+$  extrusion proceeds at a rate that is higher than the rate at which  $H^+$  can be replenished by cell metabolism and translocation across the basolateral cell membranes, then cell  $[H^+]$ , and thus  $J_H$ , should decrease until the fluxes of  $H^+$  equivalents across the basolateral and luminal cell membranes are equal.<sup>3</sup> We conclude, on the basis of the absence of an overshoot in the presence of 1%  $CO_2$ , taken in conjunction with the pro-

<sup>3</sup> Similar transients in response to acute luminal pH changes have been described for the urinary bladder of the Brazilian toad by Furtado et al. (1981).

nounced overshoot observed in the absence of exogenous CO<sub>2</sub>, that the basolateral cell membrane has a high permeability to H<sup>+</sup> equivalents in the presence of 1% CO<sub>2</sub>, and that this membrane cannot be rate-limiting for the transepithelial transport of H<sup>+</sup> under our experimental conditions.

*The Relation Between  $J_H$  and  $\Delta\bar{\mu}_H$  Reflects the Intrinsic Properties of the H<sup>+</sup> Pump*

The similar transport behavior observed in the absence and presence of colchicine (Figs. 1 and 3) and the virtual absence of an overshoot in  $J_H$  in the presence of CO<sub>2</sub> (Fig. 2) suggest that vesicle fusion and membrane retrieval or intracellular pH changes are of relatively little importance for the relation between  $J_H$  and luminal pH and, consequently, that the saturating  $J_H$  vs.  $\Delta$ pH tracings reflect the intrinsic characteristics of the H<sup>+</sup> pump. The available electrophysiological

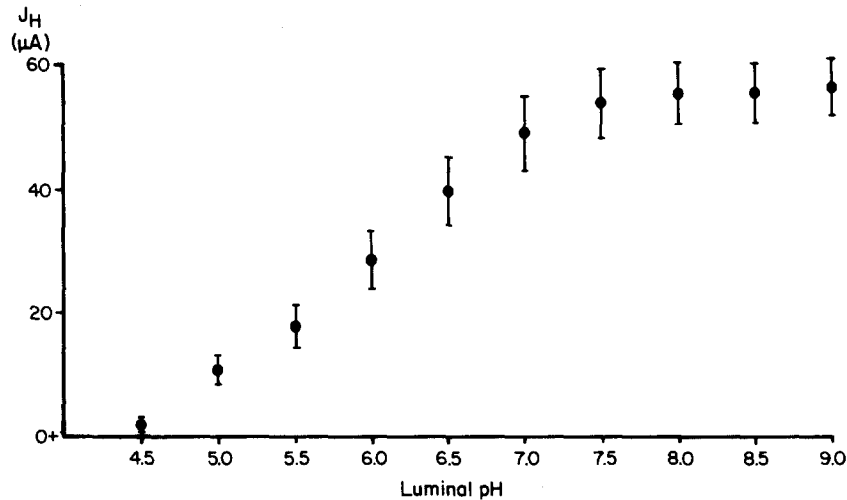


FIGURE 5. Relation between  $J_H$  and luminal pH in a group of eight bladders.  $J_H$  reaches a maximal value between pH 8 and 9.

studies on the turtle urinary bladder (Nagel et al., 1981; Clausen and Dixon, 1984) and recent studies on the inner stripe of the outer medullary collecting duct (Koeppen, 1985) indicate further that the electrical resistance of the luminal membrane of proton-secreting tight urinary epithelia is 20–100 times higher than the resistance of the basolateral membrane. A transepithelial  $\Delta\psi$  should thus be expressed almost completely as a change in  $\Delta\psi$  across the luminal cell membrane, and the intracellular [H<sup>+</sup>] should be relatively invariant with respect to changes in both  $\Delta$ pH and  $\Delta\psi$ . A further exploration of the effects of  $\Delta$ pH and  $\Delta\psi$  on  $J_H$  may therefore provide insight into the mechanism of H<sup>+</sup> translocation through the pump.

The relation between  $J_H$  and luminal pH is illustrated in Fig. 5. In accord with previous studies (Al-Awqati et al., 1977),  $J_H$  was a near-linear function of luminal pH between 4.5 and 7.0. At higher pH values, however,  $J_H$  appears to saturate,

reaching a maximal value between pH 8 and 9. In these experiments,  $J_H$  was measured as the (reverse) short-circuit current after  $\text{Na}^+$  transport was abolished with ouabain. It was not possible to use this technique to study the effect of a  $\Delta\psi$  on  $J_H$ , as extraneous currents obscure the current caused by active  $\text{H}^+$  transport. It was therefore necessary to use the more cumbersome pH-stat titration technique when a  $\Delta\psi$  was imposed across the bladder. The pH-stat experiments were done at a constant luminal pH of 7.0. This pH was chosen as a compromise between the technical demands, which were best met at a fairly low pH (a small luminal  $\text{HCO}_3^-$  pool relative to  $J_H$ ), and the interest in pursuing the  $\Delta\psi$  experi-

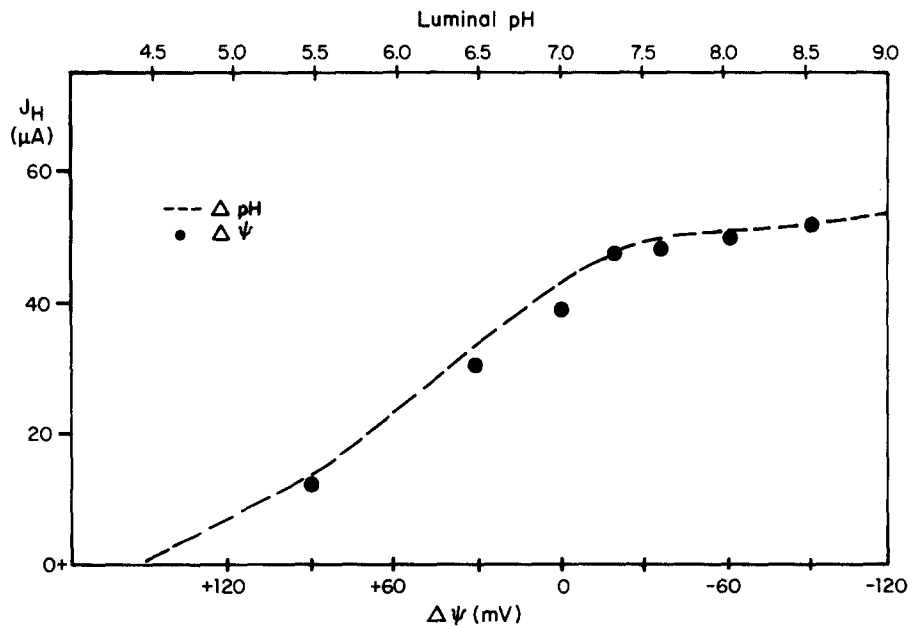


FIGURE 6. Comparison of the effects of imposed pH differences ( $\Delta\text{pH}$ ) and imposed potential differences ( $\Delta\psi$ ) on  $J_H$ . The dashed line represents the average  $J_H$  vs. pH relation for four bladders where pH-stat experiments were performed. The effects of  $\Delta\psi$  were examined at luminal pH 7.0 and at  $\Delta\psi$ 's ranging between  $-90$  and  $+90$  mV, and the resulting  $J_H$  was measured by pH-stat titration. The solid circles represent the average  $J_H$  for at least two bladders at the indicated  $\Delta\psi$ . Note that  $J_H$  was affected comparably by  $\Delta\text{pH}$  and  $\Delta\psi$ .

ments at a high pH, to see whether the maximal level of  $J_H$  in the  $\Delta\text{pH}$  experiments was affected by a transepithelial potential difference.

Fig. 6 illustrates how  $J_H$  was affected by lumen-positive and lumen-negative potentials. The  $\Delta\psi$  data follow the  $\Delta\text{pH}$  data not only in the linear region, as previously observed (Al-Awqati et al., 1977), but also in the region where  $J_H$  levels off to become constant, independent of  $\Delta\psi$ . The value of  $J_H$  at luminal pH  $\geq 8.0$  was  $52.2 \pm 2.2 \mu\text{A}$  (12 measurements), while  $J_H$  at  $\Delta\psi \geq 60$  mV was  $51.0 \pm 1.2 \mu\text{A}$  (7 measurements).



## DISCUSSION

The main results of this study are (a) that the  $J_H$  vs.  $\Delta\text{pH}$  or  $\Delta\psi$  relations are nonlinear, (b) that  $J_H$  reaches a maximal value as the lumen is made alkaline or electrically negative relative to the serosal solution, and (c) that the  $J_H$  vs.  $\Delta\text{pH}$  relation is very similar to the  $J_H$  vs.  $\Delta\psi$  relation, so that almost identical maximal values for  $J_H$  are reached under the two experimental conditions.

To facilitate the discussion, the essential steps in urinary acidification by the turtle bladder are illustrated in Fig. 7. The overall process can be described as the dissociation of  $\text{H}_2\text{O}$  into  $\text{H}^+$  and  $\text{OH}^-$ , coupled to the vectorial translocation of  $\text{H}^+$  into the luminal compartment and of  $\text{OH}^-$  into the serosal compartment. The transport processes thus occur across two membranes, and either or both may be rate-limiting for the active  $\text{H}^+$  translocation across the epithelium.

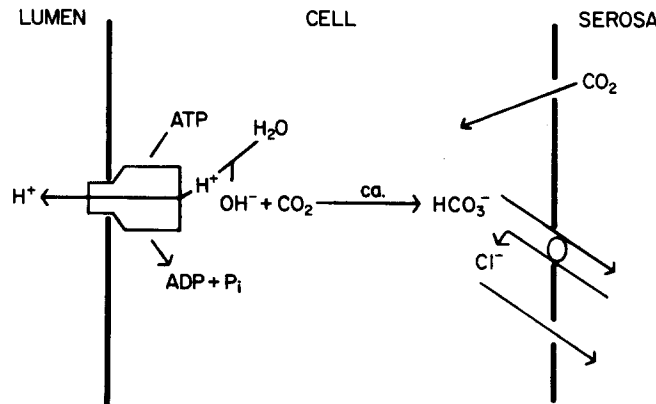


FIGURE 7. Cellular organization of  $\text{H}^+$  secretion in the CA-containing cells of the turtle bladder. The ATP-driven  $\text{H}^+$  translocation step is located in the luminal cell membrane. The  $\text{OH}^-$  exit occurs at the basolateral cell membrane, possibly as a  $\text{HCO}_3^-/\text{Cl}^-$  exchange coupled to a net  $\text{Cl}^-$  conductance.

#### *Analysis of Active $\text{H}^+$ Transport as a Single-Membrane Problem*

The proton pump is located at the luminal membrane, which has a very low passive permeability to  $\text{H}^+$ , as judged from several lines of evidence. First, the apparent epithelial pH remains fairly alkaline ( $\sim 7.4$ ) when the luminal pH is lowered to  $\sim 5.4$  (Steinmetz, 1969). Second, maneuvers that render the luminal membrane permeable to  $\text{H}^+$ , such as luminal addition of amphotericin B (Steinmetz and Lawson, 1970, 1971) or low concentrations of the protonophore 2,4-di- $\text{NO}_2$ -phenol (DNP) (Beauwens and Al-Awqati, 1976), reduce net  $\text{H}^+$  secretion only in the presence of a  $\Delta\text{pH}$  favoring back-diffusion of  $\text{H}^+$ . At  $\Delta\text{pH} = 0$ ,  $J_H$  is unaffected. Third,  $\text{H}^+$  transport is normally tightly coupled to metabolism, and DNP uncouples  $\text{H}^+$  transport from metabolism only at acid luminal pH, which indicates that DNP exerts its uncoupling effect by recycling  $\text{H}^+$  across the luminal cell membrane (Beauwens and Al-Awqati, 1976).

The luminal membrane of the turtle bladder has also a low electrical conductance relative to the basolateral membrane, although the precise electrical profile of the  $H^+$ -secreting CA cells remains unknown. Intracellular measurements (Nagel et al., 1981), presumably from the dominant population of granular cells, and impedance measurements (Clausen and Dixon, 1984) show that the resistance of the luminal membrane in the presence of 10–100  $\mu M$  amiloride is  $\sim 20$  times greater than the resistance of the basolateral membrane. Direct measurements on the CA cells have not been reported for the turtle bladder, but such measurements have been made in the  $H^+$ -secreting cells of the inner stripe of the outer medullary collecting duct (Koeppen, 1985). This tight urinary epithelium contains CA and is specialized for  $H^+$  secretion. The electrical resistance of the luminal cell membrane in this segment is  $\sim 100$  times greater than the resistance of the basolateral cell membrane, and the conductive characteristics of the basolateral membrane are similar to those depicted in Fig. 7. It is thus reasonable to assume that the luminal cell membrane of the CA cells in the turtle bladder has a much larger electrical resistance than the basolateral cell membrane.

The  $OH^-$  generated by the  $H^+$  pump (see Fig. 7) reacts with  $CO_2$  and the resulting  $HCO_3^-$  moves across the basolateral cell membrane via specific transport sites. The efflux of  $HCO_3^-$  is inhibited by 4-acetamido-4'-isothiocyano-2,2'-disulfonic stilbene (SITS) added to the serosal solution (Ehrenspeck and Brodsky, 1976; Cohen et al., 1978; Husted et al., 1979), and by removal of  $Cl^-$  from the serosal solution (Fischer et al., 1983). These studies suggest that the efflux of  $HCO_3^-$  is mediated by a  $Cl^-HCO_3^-$  exchanger that renders the basolateral membrane very permeable to  $HCO_3^-$  under control conditions. The eventual transport of negative charge outward across the basolateral cell membrane requires an additional conductance in parallel with the exchanger, e.g., a  $Cl^-$  pathway such as that illustrated in Fig. 7. Such a  $Cl^-$  conductance has been demonstrated in the  $H^+$ -secreting cells in the outer medullary collecting duct (Koeppen, 1985).

The high acid-base permeability of the basolateral cell membrane in the presence of  $CO_2$  and  $HCO_3^-$  was previously demonstrated by Cohen and Steinmetz (1980). The high permeability to  $H^+$  equivalents<sup>4</sup> depends on the presence of  $CO_2$  and  $HCO_3^-$ , because the pronounced biphasic response of  $J_H$  to an acute increase in the luminal pH occurred only in the absence of exogenous  $CO_2$  and  $CO_3^-$  (Fig. 4). There was little, if any, overshoot in the presence of 1%  $CO_2$  (Figs. 1 and 2). This implies that the permeability of the basolateral cell membrane to  $H^+$  equivalents under our experimental conditions has become sufficiently large that this membrane will not be rate-limiting for transepithelial  $H^+$  movement.

The complex structure of the turtle urinary bladder is thus largely irrelevant for the analysis of the relation between  $J_H$  and the transepithelial  $\Delta pH$  or  $\Delta\psi$ . The analysis of the active  $H^+$  transport may be simplified to an analysis of active  $H^+$  transport across a single membrane, the luminal cell membrane, uncontaminated by passive  $H^+$  leaks. For this analysis, the serosal and cellular compartments

<sup>4</sup> One can regard the process of  $CO_2$  and  $H_2O$  influx coupled to the  $HCO_3^-$  efflux as a net  $H^+$  influx.  $CO_2$  is highly permeable across the turtle urinary bladder (e.g., Steinmetz, 1974), and it is unlikely that  $CO_2$  movement will be rate-limiting for the  $H^+$  movement.

can be considered together because, although the values for the intracellular pH and the potential difference across the luminal cell membrane were not monitored directly, the imposed changes in  $\Delta\text{pH}$  or  $\Delta\psi$  were well defined and uncertainties about the exact values of  $\Delta\text{pH}$  or  $\Delta\psi$  should have little effect on our conclusions.

*A Model for Active H<sup>+</sup> Transport That Accounts for the Shape of the J<sub>H</sub> vs. ΔpH or Δψ Relations*

We have concluded that these relations reflect the intrinsic characteristics of H<sup>+</sup> transport by the H<sup>+</sup> pumps. It is therefore appropriate to examine their shapes in more detail to obtain information about the H<sup>+</sup> pump. We will to this end make use of a simple model for the H<sup>+</sup> pump (Steinmetz and Andersen, 1982). The essential features of the model are illustrated in Fig. 8A.

The pump is envisaged to be ATP-driven and to consist of two elements in series: a transmembrane channel, and a catalytic unit facing the cytoplasm. This subdivision is chosen based on the structure of the F<sub>0</sub>-F<sub>1</sub> ATP synthetases, the only ATP-driven ion pumps where significant structural information is available (e.g., Baird and Hammes, 1979; Kagawa et al., 1979). (There is, however, evidence which suggests that other ATPases exhibit a similar organization [Cantley et al., 1982; Pedersen, 1982; Repke, 1982].) It is further assumed, for simplicity, that H<sup>+</sup> translocation through each of the two subsystems is intrinsically independent of the state of the other subsystem. That is, we assume that there exists an intermediary compartment (an antechamber) with a high H<sup>+</sup> buffer capacity, which serves as a transient source (sink) for H<sup>+</sup> moving through the system. The rate of H<sup>+</sup> translocation through the channel segment is thus determined only by the [H<sup>+</sup>] in the antechamber and the luminal solution, the  $\Delta\psi$  across the channel, and its intrinsic permeability properties. The rate of H<sup>+</sup> translocation through the catalytic unit is similarly determined only by the [H<sup>+</sup>] in the antechamber and cytoplasm, the electrical potential difference across this element, the rate constants governing the H<sup>+</sup> translocation through it, and the cellular concentrations of ATP, ADP, and inorganic phosphate, P<sub>i</sub>. It is further assumed that changes in the transepithelial  $\Delta\psi$  are expressed fully as changes in the transmembrane potential difference across the luminal membrane, and that [H<sup>+</sup>]<sub>c</sub> is invariant with respect to changes in luminal pH (transepithelial  $\Delta\text{pH}$ ) or  $\Delta\psi$ . H<sup>+</sup> translocation through the catalytic unit can be described by a kinetic scheme similar to that used to describe a variety of active transport systems (Shaw, 1954; Patlak, 1957; Finkelstein, 1964; Boyer, 1975; Lauger, 1979; Hansen et al., 1981) (see Fig. 8B). This model is of necessity simplified and is almost certainly incomplete. Because of its structural features, however, it lends itself to a fairly immediate and general interpretation of the superposition of the J<sub>H</sub> vs.  $\Delta\text{pH}$  or  $\Delta\psi$  relations, and the existence of a maximal value for J<sub>H</sub> when the lumen is made alkaline or electrically negative relative to the serosal solution.

The maximal values for J<sub>H</sub> in  $\Delta\text{pH}$  or  $\Delta\psi$  experiments are reflections of the finite time it takes to complete a full rotation through the kinetic transitions for the catalytic unit. A maximal value for J<sub>H</sub> in  $\Delta\text{pH}$  experiments (at a constant [H<sup>+</sup>]<sub>c</sub>) is probably a general feature of H<sup>+</sup>-ATPases and other ion pumps (e.g.,

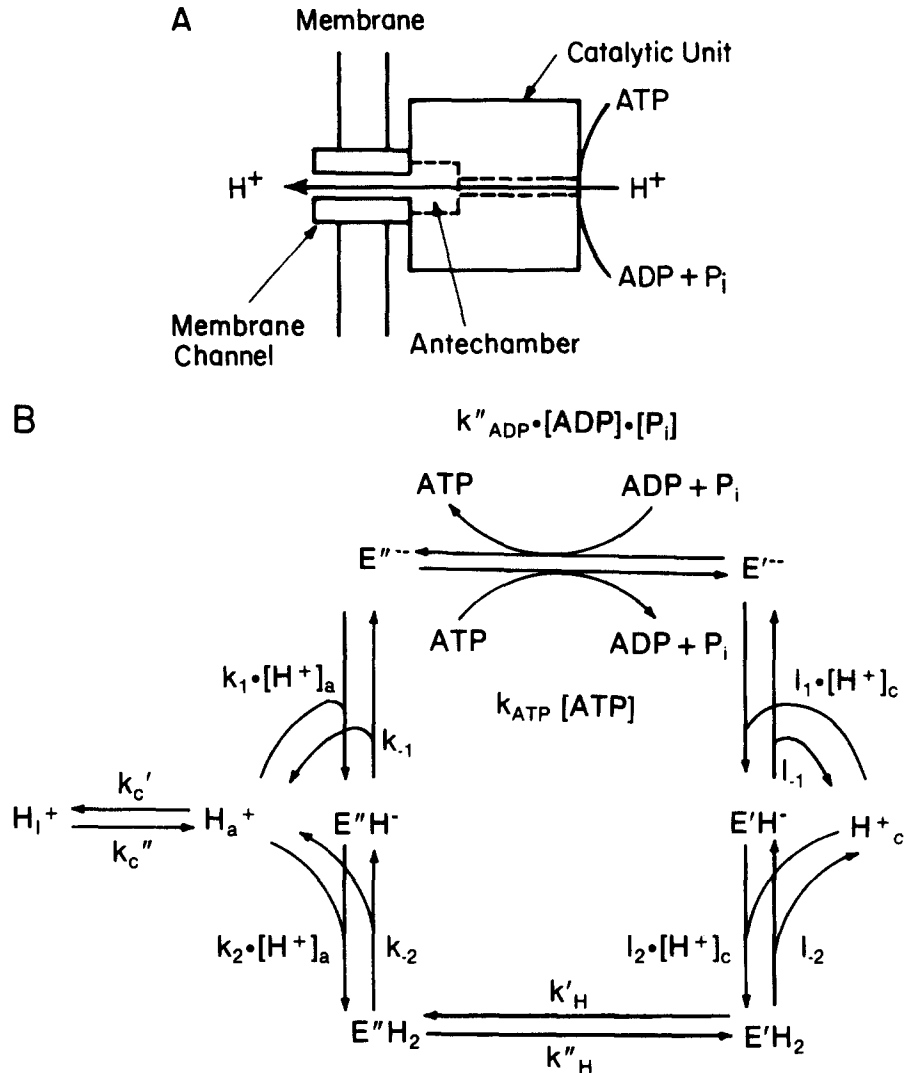


FIGURE 8. (A) Schematic representation of an ATP-driven proton pump based upon the structure of the  $F_0$ - $F_1$  ATP synthetases. The pump consists of three components: a membrane channel, an antechamber that serves as buffer compartment, and a catalytic unit where ATP hydrolysis is coupled to  $H^+$  translocation. The path for  $H^+$  translocation through the catalytic unit is represented by the dashed lines. (B) Reaction scheme for active  $H^+$  translocation through the proton pump. The horizontal segment to the left represents  $H^+$  translocation through the transmembrane channel, connecting the antechamber with the luminal solution with rate constants  $k'_c$  and  $k''_c$ , respectively. The rectangular segment to the right represents the reaction sequence in the catalytic unit. The catalytic unit is considered to exist in six states:  $E''^-$ ,  $E'H^-$ ,  $E'H_2$ ,  $E''^-$ ,  $E''H^-$ , and  $E''H_2$ . The kinetic transitions interconverting these states are depicted in the figure. Note that the kinetic transitions through the two segments are separate, albeit coupled through  $[H^+]_a$ , the  $[H^+]$  in the antechamber.  $[H^+]_l$  and  $[H^+]_c$  denote the  $H^+$  concentrations in the

Hansen et al., 1981), since the translocation rate from cell to lumen will reach a limit when  $[H^+]_l = 0$ , where  $J_H$  becomes equal to the unidirectional flux of  $H^+$  from cell to lumen. The magnitude of this limit depends upon the intracellular concentrations of  $H^+$ , ATP, ADP, and  $P_i$ , and on the intrinsic rate constants for translocation. A maximal value for  $J_H$  in  $\Delta\psi$  experiments is, on the other hand, a fairly specific finding (even though it may be a general feature of ion pumps). Two explanations can be considered for the voltage insensitivity of  $J_H$  observed at a  $\Delta\psi$  of less than  $-60$  mV: either one of the rate constants in the reaction sequence must be insensitive to changes in potential, because no charge is moved in one of the steps (e.g., the  $E'H_2$  to  $E'H_2$  transition in Fig. 8B), or the applied potential difference is distributed almost entirely across the channel segment, and will therefore not affect the catalytic unit directly. Either or both possibilities could apply in the present situation.

The superposition of the ion transport rate vs.  $\Delta\mu$  or  $\Delta\psi$  ( $J_H$  vs.  $\Delta pH$  or  $\Delta\psi$ ) relations over a large range of  $\Delta\mu$  ( $\Delta pH$ ) or  $\Delta\psi$ , as well as the extended linear regions in these relations (Figs. 5 and 6), are common features of electrogenic transport systems (Ussing and Zerahn, 1951; Danisi and Viera, 1974; Caplan and Essig, 1977; Heinz and Geck, 1978; Essig and Caplan, 1981; Hansen et al., 1981; Steinmetz and Andersen, 1982). These features have been fairly resistant to kinetic modeling, although it is possible to simulate them under somewhat restrictive conditions (Hansen et al., 1981). The present model lends itself to a direct and general interpretation of these observations. The very similar maximal values for  $J_H$  in the  $\Delta\psi$  and  $\Delta pH$  experiments suggest that the rate-limiting step in the overall  $H^+$  translocation is the same in the two experimental situations. This result indicates, therefore, that there is no appreciable potential difference (associated with the applied potential) across the segments of the transport system that contribute to the rate-limiting step(s), or that no net charge is transferred in the rate-limiting step(s). Most of an applied potential difference must fall across the luminal cell membrane and the transmembrane channel segment. In the context of the working model (Fig. 8A), the results thus demonstrate that the intramembraneous channel ( $F_0$ ) segment cannot be rate-limiting for  $H^+$  translocation.<sup>5</sup>

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luminal and intracellular solutions. [ATP], [ADP], and [ $P_i$ ] denote the cellular concentrations of these substances. The dissociation constants for  $H^+$  binding to  $E'^{-}$ , etc., are denoted as  $L_1 = l_1/l_1$ , etc. Any effect of an applied transmembrane potential on the translocation rate constants through the catalytic unit are incorporated into the rate constants ( $k'_c$ ,  $k''_c$ , and possibly  $k'_{ATP}$  and  $k''_{ADP}$  in the present model; in the absence of a transmembrane potential difference, the latter two rate constants will be denoted by unprimed symbols).

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<sup>5</sup> The distribution of the applied potential difference between the channel segment and catalytic unit does not reflect the relative resistances to  $H^+$  translocation imposed by these components of the  $H^+$  pump. This perhaps counterintuitive situation arises because the rate of  $H^+$  translocation is determined by the energy profile for  $H^+$  movement through the pump (Lauger, 1979), where the total energy profile for a permeating  $H^+$  results from the superposition of the applied potential profile with the other contributions to the potential energy (e.g., Andersen, 1978).

If the distribution of  $H^+$  between the luminal solution and the antechamber is in equilibrium, and if all of the applied potential difference falls across the transmembrane channel, a mechanism exists to equate the chemical ( $-RT \ln 2.303 \Delta pH$ ) and the electrical ( $F\Delta\psi$ ) contributions to  $\Delta\tilde{\mu}_H$  with respect to their kinetic effects. The  $[H^+]$  in the antechamber,  $[H^+]_a$ , can be expressed as:

$$[H^+]_a = [H^+]_i K_{tmc} \exp(F\Delta\psi/RT), \quad (1)^6$$

where  $[H^+]_i$  is the luminal  $[H^+]$ ,  $K_{tmc}$  is the distribution coefficient for  $H^+$  between the luminal solution and the antechamber when  $\Delta\psi = 0$  [ $\tilde{\mu}_H^0(\text{lumen}) - \tilde{\mu}_H^0(\text{antechamber}) = RT \ln(K_{tmc})$ ], where  $\tilde{\mu}_H^0$  denotes the standard chemical potential for  $[H^+]$ ,  $F$  is the Faraday constant,  $R$  is the gas constant, and  $T$  is the absolute temperature. The relevant  $[H^+]$  for the catalytic ( $F_1$ ) segment is  $[H^+]_a$ , which now reflects both  $\Delta pH$  ( $[H^+]_i$ ) and  $\Delta\psi$  in a combination where the two components of  $\Delta\tilde{\mu}_H$  are indistinguishable in terms of their effect on the kinetics of  $H^+$  translocation.

The kinetic scheme in Fig. 8B was solved subject to the assumption of equilibrium distribution of  $H^+$  between the two ends of the transmembrane channel (see the Appendix). The resulting expression for  $J_H$  is:

$$J_H = 2(k_{\text{forward}}[H^+]_c^2 - k_{\text{backward}}\exp(2F\alpha\Delta\psi/RT)[H^+]_i^2)/D, \quad (2)$$

where  $\alpha$  is the fraction of  $\Delta\psi$  that drops across the transmembrane channel segment ( $0 \leq \alpha \leq 1$ ), while  $k_{\text{forward}}$ ,  $k_{\text{backward}}$ , and  $D$  are given by:

$$k_{\text{forward}} = k'_H k'_{ATP} [ATP] K_1 K_2, \quad (3)$$

$$k_{\text{backward}} = k''_H k''_{ADP} [ADP] [P_i] L_1 L_2 (K_{tmc})^2, \quad (4)$$

and

$$D = k'_{ATP} [ATP] f_c([H^+]_c, [H^+]_i, \Delta\psi) + \quad (5)$$

$$k''_{ADP} [ADP] [P_i] f_a([H^+]_c, [H^+]_i, \Delta\psi) + f_H([H^+]_c, [H^+]_i, \Delta\psi),$$

where the functions  $f_c$ ,  $f_a$ , and  $f_H$  are defined in the Appendix. The applied potential enters through Eq. 1 ( $f_c$ ,  $f_a$ , and  $f_H$ ) and, if  $\alpha < 1$ , the voltage dependence of  $k'_{ATP}$  and  $k''_{ADP}$ , which for the present purposes can be represented as symmetrical (Eyring-type) barriers (e.g., Lauger and Stark, 1970). Eq. 2 can provide a reasonable fit to the experimental data on  $H^+$  transport (see Fig. 9).

The curve in Fig. 9 is based on one of many possible sets of parameters describing the behavior of the catalytic unit, which give an equally good simulation of the data. There is at present no basis for choosing one parameter set over another, but we note that the superposition of the  $J_H$  vs.  $\Delta pH$  or  $\Delta\psi$  relations is a feature of the structure of the model, independent of the kinetic characteristics of  $H^+$  translocation through the catalytic unit. It seems, however, to be characteristic that the rate constant for the binding of the second  $H^+$  at the "exterior"

<sup>6</sup> If all of the transepithelial  $\Delta\psi$  (which is assumed to drop across the luminal cell membrane) does not drop across the transmembrane channel itself, it becomes necessary to consider this as in Eq. A16 in the Appendix.

surface of the catalytic unit must be comparable to or smaller than the corresponding rate constant for the first  $H^+$  (with the inverse relation for the dissociation rate constants), such that the shape of the  $J_H$  vs.  $\Delta pH$  or  $\Delta\psi$  relations approximate simple monovalent titration curves. Indeed, one cannot on the basis of the shape of the  $J_H$  vs.  $\Delta pH$  or  $\Delta\psi$  relations determine the  $H^+/ATP$  stoichi-

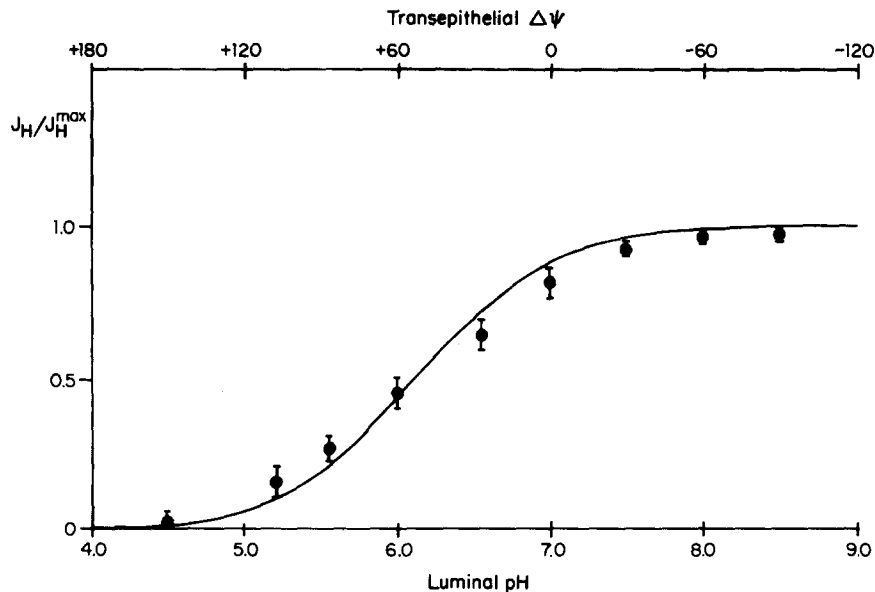


FIGURE 9. Simulation of  $J_H$  vs.  $\Delta\tilde{\mu}_H$  characteristics. The closed circles represent the experimental data for the  $J_H$  vs.  $\Delta pH$  relation from Fig. 5 normalized by  $J_H$  at luminal pH 9.0. The full curve depicts a simulation of a  $\Delta pH$  or a  $\Delta\psi$  experiment with the following parameter values:  $K_1 = 3$ ,  $L_1 = 1$ ,  $K_2 = 30$ ,  $L_2 = 0.25$ ,  $[H^+]_c = 1$ , where the dissociation constants and  $[H^+]$  have been normalized relative to one another (for comparison with the experimental data, the intracellular pH is assumed to be 7.5); the transmembrane potential across the luminal cell membrane is assumed to be 30 mV (lumen-positive) in the  $\Delta pH$  simulation, while the luminal pH is 7.0 in the  $\Delta\psi$  simulation, and  $\alpha = 1.0$ ;  $\Delta G_{ATP}^\circ = -33$  kJ/mol,  $[ATP] = 2.5 \times 10^{-3}$  M, and  $[ADP][P_i] = 10^{-7}$  M<sup>2</sup>, i.e.,  $[ATP]/([ADP][P_i]) = 25,000$  M<sup>-1</sup> (Veech et al., 1979);  $k_{-1} = l_{-1} = 10^3$  s<sup>-1</sup>,  $k_{-2} = 10^4$  s<sup>-1</sup>,  $l_{-2} = 250$  s<sup>-1</sup>;  $k_{ATP} = 10^9$  M<sup>-1</sup>s<sup>-1</sup>,  $k_{ADP} = 8.2 \times 10^4$  M<sup>-2</sup>s<sup>-1</sup>;  $k'_H = 100$  s<sup>-1</sup>,  $k''_H = 500$  s<sup>-1</sup>;  $K_{tmc} = 1$ . The simulations are normalized by  $J_H^{max}$ ,  $J_H$  at a luminal pH of 9.0 or at a  $\Delta\psi$  of -120 mV, respectively. The abscissa denotes the luminal pH and the transepithelial  $\Delta\psi$ . Note that the current becomes indistinguishable from 0 at a luminal pH  $\sim 4.3$ , while the thermodynamic reversal ( $J_H = 0$ ) first will occur at a luminal pH of  $\sim 3.3$ , outside the range illustrated in the figure.

ometry of the transport system; it has to be determined independently. It is in this respect important that the extrapolated reversal potential for the pump may be quite far from the thermodynamic reversal potential (see the legend to Fig. 9 and Steinmetz and Andersen, 1982). It should further be noted that the proposed model for the catalytic unit is but one of a class of models of about equal

complexity that are reversible, tightly coupled ATPases with a stoichiometry of  $2H^+/ATP$  and that display an appropriate  $J_H$  vs. luminal pH dependence. It is possible to devise simpler models that, given the structural constraints in Fig. 8A, can simulate the shape of the  $J_H$  vs.  $\Delta pH$  or  $\Delta\psi$  relations, but they do not provide an enzymologically satisfactory picture of the operation of the  $H^+$  pump.

The imperfections of this very simple model are therefore not too debilitating, and the experimental observations on the saturation of  $J_H$  at favorable  $\Delta\tilde{\mu}_H$  and the kinetic equivalence of the chemical ( $\Delta pH$ ) and electrical ( $\Delta\psi$ ) contributions to  $\Delta\tilde{\mu}_H$  over a wide range of  $\Delta\tilde{\mu}_H$  provide strong support for the view that the pump consists of two components, a catalytic unit and a transmembrane channel, which may be linked by an antechamber or buffer compartment. Further refinement (or rejection) of the model must await more detailed biochemical and structural information about the pump.

#### APPENDIX

Let  $W'$ ,  $W'_H$ ,  $W'_{H_2}$ ,  $W''$ ,  $W''_H$ , and  $W''_{H_2}$  denote the probability that the catalytic unit exists in states  $E'^{-}$ ,  $E'H^-$ ,  $E'H_2$ ,  $E''^{-}$ ,  $E''H^-$ ,  $E''H_2$ , respectively (see Fig. 8B). The transitions among these six states are described by:

$$\frac{dW'}{dt} = -(l_1[H^+]_c + k''_{ADP}[ADP][P_i])W' + l_1W'_H + k'_{ATP}[ATP]W''; \quad (A1)$$

$$\frac{dW'_H}{dt} = -(l_1 + l_2[H^+]_c)W'_H + l_1[H^+]_cW' + l_{-2}W'_{H_2}; \quad (A2)$$

$$\frac{dW'_{H_2}}{dt} = -(l_2 + k'_H)W'_{H_2} + l_2[H^+]_cW'_H + k''_HW''_{H_2}; \quad (A3)$$

$$\frac{dW''}{dt} = -(k_1[H^+]_a + k'_{ATP}[ATP])W'' + k_{-1}W''_H + k''_{ADP}[ADP][P_i]W'; \quad (A4)$$

$$\frac{dW''_H}{dt} = -(k_{-1} + k_2[H^+]_a)W''_H + k_1[H^+]_aW'' + k_{-2}W''_{H_2}; \quad (A5)$$

$$\frac{dW''_{H_2}}{dt} = -(k_{-2} + k''_H)W''_{H_2} + k_2[H^+]_aW''_H + k'_HW''_{H_2}; \quad (A6)$$

and

$$W' + W'_H + W'_{H_2} + W'' + W''_H + W''_{H_2} = 1, \quad (A7)$$

which are solved assuming the system is stationary:

$$\frac{dW'}{dt} = \frac{dW'_H}{dt} = \frac{dW'_{H_2}}{dt} = \frac{dW''}{dt} = \frac{dW''_H}{dt} = \frac{dW''_{H_2}}{dt} = 0.$$

The solution to this set of equations is trivial, except for one problem: the value of  $[H^+]_a$  in Eq. A4 is not necessarily the same as the value of  $[H^+]_a$  in Eq. A6 (the situation is even worse in Eq. A5). This problem occurs because the  $H^+$  in the antechamber may fluctuate appreciably as a function of  $H^+$  association to and dissociation from the catalytic unit, unless the  $H^+$  permeability of the transmembrane channel is very high. Only if  $H^+$  is in equilibrium between the luminal solution and the antechamber will the  $[H^+]_a$ 's in the different equations be identical to each other. If this is assumed to be the case, then



$$J_H = 2(k_{\text{forward}}[H^+]_c^2 - k_{\text{backward}}''[H^+]_a^2)/D, \quad (\text{A9})$$

where

$$k_{\text{forward}} = k_H' k_{\text{ATP}}' [\text{ATP}] K_1 K_2, \quad (\text{A10})$$

$$k_{\text{backward}}'' = k_H'' k_{\text{ADP}}'' [\text{ADP}] [P_i] L_1 L_2, \quad (\text{A11})$$

and

$$D = k_{\text{ATP}}' [\text{ATP}] f_c([H^+]_c, [H^+]_a) + k_{\text{ADP}}'' [\text{ADP}] [P_i] f_a([H^+]_c, [H^+]_a) + f_H([H^+]_c, [H^+]_a), \quad (\text{A12})$$

where

$$f_c([H^+]_c, [H^+]_a) = K_1(L_1 + [H^+]_c)(L_2 k_H''/k_2 + L_2 K_2 + K_2 k_H'/l_2) + [H^+]_c^2(K_1 K_2 + K_1(k_H'' + k_H')/k_2 + K_2 k_H'/k_1) + [H^+]_a([H^+]_c L_2 k_H''/k_1 + [H^+]_c^2(k_H'' + k_H')/k_1) + K_1 K_2 [H^+]_c k_H'/l_1 + L_1 L_2 [H^+]_a k_H''/k_1; \quad (\text{A13})$$

$$f_a([H^+]_c, [H^+]_a) = L_1(K_1 + [H^+]_a)(K_2 k_H'/l_2 + L_2 K_2 + L_2 k_H''/k_2) + [H^+]_a^2(L_1 L_2 + L_1(k_H'' + k_H')/l_2 + L_2 k_H''/l_1) + [H^+]_c([H^+]_a K_2 k_H'/l_1 + [H^+]_a^2(k_H'' + k_H')/l_1) + K_1 K_2 [H^+]_c k_H'/l_1 + L_1 L_2 [H^+]_a k_H''/k_1, \quad (\text{A14})$$

and

$$f_H([H^+]_c, [H^+]_a) = [H^+]_a^2 [H^+]_c^2 (k_H' + k_H'') + [H^+]_c^2 K_2 k_H' (K_1 + [H^+]_a) + [H^+]_a^2 L_2 k_H'' (L_1 + [H^+]_c). \quad (\text{A15})$$

The only remaining problem is to express  $[H^+]_a$  as a function of  $[H^+]_i$  and  $\Delta\psi$ :

$$[H^+]_a = [H^+]_i K_{\text{tmc}} \exp(F\alpha\Delta\psi/RT), \quad (\text{A16})$$

where  $\alpha$  is the fraction of the transepithelial  $\Delta\psi$ , which drops across the transmembrane channel. If Eq. A16 is inserted into Eqs. A9–A15, one obtains the desired expression for  $J_H$  as a function of  $[H^+]_c$ ,  $[H^+]_i$ , and  $\Delta\psi$  (see Eq. 2 in the main text), where  $f_c([H^+]_c, [H^+]_a)$ ,  $f_a([H^+]_c, [H^+]_a)$ , and  $f_H([H^+]_c, [H^+]_a)$  have been redefined using Eq. A16 to become  $f_c([H^+]_c, [H^+]_i, \Delta\psi)$ ,  $f_a([H^+]_c, [H^+]_i, \Delta\psi)$ , and  $f_H([H^+]_c, [H^+]_i, \Delta\psi)$ , respectively, and  $k_{\text{backward}}$  is given by:

$$k_{\text{backward}} = k_{\text{backward}}'' (K_{\text{tmc}})^2, \quad (\text{A17})^7$$

and where the principle of detailed balance (Onsager, 1931) is invoked to ensure that  $J_H$

<sup>7</sup> Inspection of Eqs. 1A–6A shows that  $k_1$  and  $k_2$  only occur in combination with  $[H^+]_a$ , and vice versa. It is therefore possible to rewrite the equations using the product terms  $k_1''[H^+]_i$  and  $k_2''[H^+]_i$  instead of  $k_1[H^+]_a$  and  $k_2[H^+]_a$ . The voltage dependence of the latter products (i.e., the voltage dependence of  $[H^+]_a$ ) will in this alternative coupled scheme appear as a voltage dependence of  $k_1''$  and  $k_2''$ . This formulation will give predictions that are identical to those of Eq. 2, if the corresponding dissociation rate constants,  $k_1'$  and  $k_2'$ , are voltage-independent and identical in magnitude to their unprimed counterparts, and the magnitudes of the zero-voltage values of  $k_1''$  and  $k_2''$  are identical to those of  $k_1$  and  $k_2$ . It is therefore not necessary that the catalytic unit and the transmembrane channel be kinetically independent of each other, although the particular assignment of the voltage dependences of the  $k$ 's implies that there cannot be a significant resistance to ion movement through the channel proper—all of the resistance must reside at the channel/catalytic unit interface (e.g., Jack et al., 1975).

reverses when

$$\begin{aligned} -RT \, 2.303 \, \Delta\text{pH} + F\Delta\psi &= -\Delta G_{\text{ATP}}/2 \\ &= -\{\Delta G_{\text{ATP}}^{\circ} + RT \ln([ADP][P_i]/[ATP])\}/2, \end{aligned} \quad (\text{A18})$$

where  $\Delta G_{\text{ATP}}$  and  $\Delta G_{\text{ATP}}^{\circ}$  denote the Gibbs free energy and standard free energy for ATP hydrolysis. Eq. A18 implies that (at  $\Delta\psi = 0$ ):

$$\Delta G_{\text{ATP}}^{\circ} = RT \ln[k'_1 k''_{\text{ADP}} L_1 L_2 K_{\text{imc}}^2 / (k'_1 k'_{\text{ATP}} K_1 K_2)]. \quad (\text{A19})$$

In other cases, where  $\text{H}^+$  is not in equilibrium distribution across the channel, one must treat the variations in  $[\text{H}^+]_a$  exactly. This will lead to considerably more tedious solutions (e.g., Northrup et al., 1982; Szabo et al., 1982), unless  $[\text{H}^+]_a$  is sufficiently large that the concentration fluctuations are insignificant, or the  $\text{H}^+$  association reactions are cooperative such that they can be described as the simultaneous reaction of two  $\text{H}^+$  with the catalytic unit (e.g., Steinmetz and Andersen, 1982).

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